

In vitro Anthelmintic Activity of Different Extracts of *Memecylon umbellatum* Burm.

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Abstract	Article Information
<p>In the present study, the possible anthelmintic effects of different extracts (petroleum ether, chloroform, and ethanol) and two isolated compounds β-stigmasterol (PI) and Sitosterol (PII) from the leaves of <i>Memecylon umbellatum</i> on Indian earthworms <i>Pheretima Posthuma</i> was investigated by <i>in vitro</i> experiments. Various concentrations (25, 50, 100mg/ml) of all extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. From the activity results it was found that, the ethanolic extract and pure compound PI having the equipotent activity with standard drug albendazole. Form the observations made higher concentration of extract produced paralytic effect much earlier and the time to death was shorter for all worms.</p> <p>Copyright©2015 STAR Journal, Wollega University. All Rights Reserved.</p>	<p>Article History: Received : 12-11-2015 Revised : 19-12-2015 Accepted : 23-12-2015</p> <p>Keywords: <i>Memecylon umbellatum</i> <i>Pheretima Posthuma</i></p> <p>*Corresponding Author: Krishnappa M E-mail: krishnappam4281@yahoo.com</p>

INTRODUCTION

The word Helminth has been derived from the Greek which means worm (Bhatia, 2002). The World Health Organization estimates that a staggering two billion people harbor parasitic worm infections. Helminths are mostly present in the human intestines either in large or small intestine and produce infections. Such types of infestation produced by worms are called as Helminthiasis (Archar, 1985 and Satoskar, 2009). Among the animal diseases helminthiasis is the most important inflicting heavy production losses. In developing countries helminthes are the most common infectious agents of humans they produce a global burden of disease and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia. Particularly in third world countries due to poor management practices this disease is highly prevalent (Dhar *et al.*, 1982). The proposal of screening medicinal plants for their anthelmintic activity was led by increasing problems of development of resistance in helminthes (Geert and Dorny, 1995; Coles, 1997). Many researchers investigated that the plants are known to provide a rich source of botanical anthelmintics (Satyavati *et al.*, 1976; Lewis and Elvin-Lewis, 1977). The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases (Sondhi, 1994). Therefore there is an increasing demand towards natural anthelmintics. The parasites can be acquired by contact with a) infected water b) infected meal c) infected animal. The earlier studies of many workers confirmed that number of medicinal plants have been used to treat parasitic infections in man and animals (Chopra *et al.*, 1956; Akhtar *et al.*, 2000; Iqbal *et al.*, 2004). The practitioners in traditional system of medicine use various

indigenous plants for the treatment of anthelmintic. Considerable hardship and stunned growth of animals and men was caused by parasitic helminthes affects. Helminthes probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. Most diseases caused by helminthes are of a chronic; debilitating nature. The use if anthelmintins is the major control strategy adopted against helminthes parasite (Kapoor, 2000).

Memecylon umbellatum Burm. belongs to the family Melastomaceae is a small handsome tree, the local name is Adachare and the English name is Iron-wood tree. The infusion of leaves is used in the treatment of gonorrhoea and leucorrhoea (Nadakarni, 1976; Kirtikar and Basu, 1991). The decoction of the roots is used for the treatment of menorrhagia and the preparation from the bark for the treatment of bruises (Nadakarni, 1976). The seeds are used to cure cough and sedative (Balakrishna Gowda, 2004). It has hypoglycemic effect in normal and alloxan diabetic mice (Amalraj and Ignacimuttu, 1998). Krishnamurthy and Asha (2010) determined the chemical composition of *M. umbellatum* with respect to proximates, nutritive value and elemental composition from the two distinct climatic regions of the Karnataka for both young and mature leaves and reported that young leaves are more nutritive. Puratchikody and Nagalakshmi (2007) evaluated the wound healing activity of alcoholic extract of leaves in rats in the form of ointment. The leaves has been being used for the preparation of herbal formulation (Kirtikar and Basu 1991, Rajakumar and Shivanna, 2009, Ayyanar *et al.*, 2008, Maruthi *et al.*, 2000, Kshirsagar and

Singh, 2001). The present study investigated the anthelmintic activity of the leaves of *M. umbellatum* for the different extracts and two pure compounds with view to justify the use of plant in the treatment of helminths.

MATERIALS AND METHODS

Plant Materials and Preparation of Extracts

The leaves of *Memecylon umbellatum* was collected from Narasimharajapura taluk of Chikmaglore district, Karnataka, India. The taxonomic identification was carried out with the help of available literature and herbaria were prepared by following standard methods and maintained in the department of Applied Botany, Kuvempu University (Specimen voucher No. KU/AB/BA-4). Air shade dried and pulverized plant material was used. Extracts were prepared using soxlet apparatus with different solvents (chloroform, petroleum ether and ethanol). Vacuum dried extracts are used for the experiment. Along with crude extracts the pure compounds β -stigmaterol (PI) and Sitosterol (PII) were also taken for the study.

Anthelmintic Bioassay

Healthy adult Indian earthworms, *Pheretima postuma*, (Annelida, Megescolecidae) were used in the present study due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings (Vidyarthi, 1967 and Thorn *et al.*, 1977). They were procured from local supplier at Shimoga at the time of carrying out the experiment. The worms were washed with water to remove adhering materials and were sorted out for uniform size and length. The worms were kept in 6% dextrose solution for acclimatization. The worms with normal motility were selected for the experiment. 25ml of physiological solution was poured into Petri dishes. Three worms of about the same size per Petri dish were used. The anthelmintic activity was determined in duplicate. They were observed for their spontaneous motility and evoked responses. The paralytic score was recorded at different time intervals. Immediate after inhibition of response to external stimuli, the worms were placed in fresh water and observed for recovery. Duration required for final recovery or death was noted. On the basis of available literature we have chosen Albendazole as reference standard. Determination of time of paralysis and time of death of the worm were done at room temperature. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C) followed with fading away of their body colours (Kelly and Hall, 1979; Dash *et al.*, 2002).

Assessment of Anthelmintic Activity

The anthelmintic assay was carried as per the literature method (Ajaiyeoba *et al.* 2001) with minor modifications.

Study Protocol: Seven groups of approximately equal size earthworms consisting of six earthworms in each group were used for the present study.

Group 1: Control (normal saline)

Group 2: Standard (Albendazole - 10mg/ml)

Group 3: Petroleum ether extract of different

concentration (25, 50 and 100 mg/ml)

Group 4: Chloroform extract of different concentration (25, 50 and 100 mg/ml)

Group 5: Ethanol extract of different concentration (25, 50 and 100 mg/ml)

Group 6: PI of different concentration (25, 50 and 100mg/ml) and

Group 7: PII of different concentration (25, 50, and 100 mg/ml)

RESULTS

Successive extract of leaves of *M. umbellatum* showed significant anthelmintic activity on selected Indian earth worms *Pheretima postuma*. The three crude (petroleum ether, chloroform and ethanol) extracts and two pure compounds were used for the study. However, when observed the response of worms in case of paralysis, there was significant variation among the results produced by the different extracts at different concentrations like 25, 50 and 100 mg/ml. Petroleum ether and pure compound PII showed moderate activity where as chloroform extract showed less activity (table). The increase in the concentration of the extracts showed decrease in the paralysis and death time of the worms. The ethanol extract and PI compound showed an excellent potency possessing 29.66 ± 0.66 as the paralysis time and 42.33 ± 1.45 as the death time and 25.33 ± 1.76 as the paralysis time and 46.33 ± 1.45 as the death time respectively.

The petroleum ether extract at concentration 25, 50 and 50mg/ml shows paralysis at 35.66 ± 0.33 , 34.33 ± 0.66 and 29.33 ± 0.33 min and death at 89.00 ± 3.78 , 47.66 ± 0.88 and 42.66 ± 0.33 min respectively. The chloroform extract at concentration 25, 50 and 50mg/ml shows paralysis at 40.33 ± 0.88 , 56.00 ± 1.52 and 58.00 ± 1.15 min and death at 78.33 ± 3.75 , 95.66 ± 2.33 and 92.66 ± 1.45 min respectively. The ethanol extract at concentration 25, 50 and 50mg/ml shows paralysis at 29.66 ± 0.66 , 48.00 ± 1.15 and 33.33 ± 2.60 min and death at 42.33 ± 1.45 , 53.33 ± 1.45 and 45.66 ± 2.33 min respectively. The pure compound PI at concentration 25, 50 and 50mg/ml shows paralysis at 48.00 ± 1.15 , 26.66 ± 1.79 and 25.33 ± 1.76 min and death at 45.66 ± 2.96 , 35.00 ± 2.88 and 46.33 ± 1.45 min respectively.

The pure compound PII at concentration 25, 50 and 50mg/ml shows paralysis at 42.33 ± 0.33 , 47.66 ± 1.45 and 40.66 ± 1.76 min and death at 94.66 ± 2.90 , 65.33 ± 2.90 and 67.33 ± 1.76 min respectively. In comparison with the standard drug albendazole from the observations all the samples showed variable paralysis and death times at different concentrations and the mean time values were calculated. Albendazole showed paralysis at 60.40 ± 0.02 min and death time after 120.00 ± 0.02 min. The ethanolic extract and pure compound PI having the equipotent activity with standard drug albendazole. Form the observations made higher concentration of extract produced paralytic effect much earlier and the time to death was shorter for all worms.

DISCUSSION

The drug Albendazole, is effective in a broad range of helminth infections, including round worms, hookworms, whipworms, pinworms and animal side effects. The efficacy of the drug is poorly absorbed and depends on transit time in the gastrointestinal tract. The drug has not been studied in children under two years of age. The toxicity of the drug is extremely low. During expulsion of the worms Gastro intestinal disturbances

may occur the mechanism of action involves inhibition of the glucose uptake system leading to a lethal

depletion of energy reserves in the helminthes (Sucheta *et al.*, 2011).

Table 1: Anthelmintic activity of different plant extracts and isolated compounds

Group	Treatment	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
1	Control	-	-	-
2	Standard Albendazole	25	60.40±0.02	120.40±0.02
3	Petroleum ether	25	35.66±0.33	89.00±3.78
		50	34.33±0.66	47.66±0.88
		100	29.33±0.33	42.66±0.33
4	Chloroform	25	40.33±0.88	78.33±3.75
		50	56.00±1.52	95.66±2.33
		100	58.00±1.15	92.66±1.45
5	Ethanol	25	29.66±0.66	42.33±1.45
		50	48.00±1.15	53.33±1.45
		100	33.33±2.60	45.66±2.33
6	β-stigmasterol (PI)	25	48.00±1.15	45.66±2.96
		50	26.66±1.79	35.00±2.88
		100	25.33±1.76	46.33±1.45
7	Sitosterol (PII)	25	42.33±0.33	94.66±2.90
		50	47.66±1.45	65.33±2.90
		100	40.66±1.76	67.33±1.76

± Shows mean and standard error

The effect of extracts on the paralysis (or) helminthiasis of the worm, according to the results (table) may be indicated as ethanol>petroleum ether >chloroform extracts. Among the two pure compounds analyzed PI showed more potent activity than PII. The results obtained in the present study are in accordance with the earlier studies of Sucheta *et al.*, (2011); Jitendra *et al.*, (2010); Arasan *et al.* (2010) and Vidyadhar *et al.* (2010) in which the ethanol extract showed significant anthelmintic activity against tested earth worms.

The phytochemical studies of the extracts of *M. umbellatum* showed the presence of tannins, flavonoids, phenols, steroids, saponins (Krishnamurthy and Asha, 2011). The presence of all these phytochemicals may influence the anthelmintic activity of the crude extracts. The pure compound PI and PII are phytosterols which may also involve in the anthelmintic activity of these compounds against selected earth worms.

Tannins are polyphenolic compounds (Bate-Smith, 1962). Some synthetic phenolic anthelmintics, e.g. niclosamide, oxiclozanide, bithionol, nitroxylin, etc, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1997). Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal (Athnasiadou, 2001) or glycoprotein on the cuticle of the parasite (Thompson and Geary, 1995) and cause death. Tannin containing plants increase the supply and absorption of digestible protein by animals (Waller *et al.*, 2001). This is achieved by formation of protein complexes in the rumen by tannins, which later dissociate at low pH in the abomasum to release more protein for metabolism in the small intestines of ruminant animals (Wang *et al.*, 1994). In addition, tannins or their metabolites have a direct effect on the viability of the preparasitic stages of helminths. Other phytochemicals reported to have an anthelmintic effect include essential oils (Pessoa *et al.*, 2002), flavonoids and terpenoids (Lahlou, 2002).

The possible mechanism of the anthelmintic activity of *M.umbellatum* cannot be explained on the basis of our present results. However, it may be due to its effect on inhibition of glucose uptake in the parasites and depletion of its glycogen synthesis (Singh *et al.*, 2002 and Goodman and Gilman, 2001).

CONCLUSIONS

In the present study, the possible anthelmintic effects of different extracts (petroleum ether, chloroform, and ethanol) and two isolated compounds β-stigmasterol (PI) and Sitosterol (PII) from the leaves of *Memecylon umbellatum* on Indian earthworms *Pheretima Posthuma* was investigated by *in vitro* experiments. From the result we can conclude that the experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as anthelmintic. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for anthelmintic activity.

Conflict of Interest

Conflict of interest none declared.

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